Methods for Estimating Gamete Contributions to Orchard Seed Crops and Vegetative Lots in British Columbia

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ABSTRACT

Methods are described for estimating male and female gamete contributions from parent trees to conifer orchard seedlots[1] and to vegetative lots in British Columbia. Methods are also described for estimating male gamete contributions for species and locations where contamination from non-orchard pollen is considered significant, and for calculating seedlot statistics when combining separate seedlots.

These methods support the *Chief Forester Standards for Seed Use* (the Standards), developed under the *Forest and Range Practices Act of British Columbia*. For orchard seedlots and for vegetative lots, estimates of effective population size and genetic worth must be determined in accordance with formulae provided in the Standards. These formulae require male and female gamete contributions to be estimated for each parent tree, and from non-orchard pollen. The Standards refer to "generally accepted scientific methodology" for the estimation of male and female gamete contributions to orchard seedlots. This paper defines generally accepted methods.

A number of methods are described and formulae provided for estimating male and female gamete contributions to seed and vegetative lots. Methods vary by expected precision and by application cost. Seed orchard managers should choose the method that best meets their needs.

1 In this paper, the term seedlots will also generally refer to vegetative lots, unless specified otherwise.

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1 OVERVIEW

This paper sets out the methodology for estimating male and female gamete contributions from parent trees to conifer orchard seedlots[2] and to vegetative lots in British Columbia. It also contains methods for estimating male gamete contributions for species and locations where contamination from non-orchard pollen is considered significant, and for calculating seedlot statistics when combining separate seedlots.

The methods presented are based on current knowledge and generally accepted scientific principles (see Appendix 1 for a list of the relevant scientific literature). Methods are intended to balance the cost of implementation and the risk of sampling bias and error.

2 PURPOSE OF PAPER

The *Forest and Range Practices Act of British Columbia* is supported by the *Chief Forester Standards for Seed Use* (the Standards). The Standards set out the legal requirements for the collection, registration, transfer, and use of tree seed on Crown land as of April 2005. For orchard seedlots and for vegetative lots, estimates of effective population size and genetic worth must be determined in accordance with formulae provided in the Standards. These formulae require male and female gamete contributions to be estimated for each parent tree, and from non-orchard pollen. The Standards refer to "generally accepted scientific methodology" for the estimation of male and female gamete contributions to orchard seedlots. The purpose of this paper is to define generally accepted methods.

3 CONCEPTUAL FRAMEWORK

3.1 Sampling Error and Bias

Obtaining exact gamete counts (pollen and seed) for individual orchard clones (or families), though possible, is logistically impractical and fiscally prohibitive. Consequently, gamete contributions are estimated from pollen- and seed-cone samples, and will vary from the true (and unknown) value. The difference between the true value and the sample estimate is *error*. The more accurate the estimate, the smaller the error associated with this estimate.

The amount of error varies with sample size, method of sampling, and the skill applied to sampling. Errors associated with estimates of female gamete contribution are relatively small, and derive from assumptions or imperfect measurements and assessments (e.g., visual estimate of volume of cones on a tree). Errors associated with estimates of male gamete contribution are relatively large, and also derive from assumptions (e.g., the number of ramets of each parent tree is proportional to the gamete contribution of each parent tree) or imperfect assessments (e.g., visual estimation of the volume of pollen cones on a tree).

2 The term seedlots, in this paper, will also generally refer to vegetative lots, unless specified otherwise.

Bias occurs when estimates consistently over- or underestimate the unknown true value; that is, when the error tends to be in a particular direction most of the time. For example, incorrect sampling methods could lead to consistently overestimating (or underestimating) the number of filled seeds in a measured volume of cones. Bias is usually of more concern than error in sample estimates (although the two are related to a degree).

Generally, estimates of gamete contributions to orchard seedlots combine a relatively accurate estimate of the female component, and a relatively inaccurate estimate of the male component. Together, when proper methodology is followed, these can form an acceptable estimate of gamete contributions for the purpose of estimating statistics required for the Standards.

3.2 Potential Impact of Error and Bias in Estimates of *Ne* and *GW*

Under the Standards, gamete contribution estimates of parent trees are used to calculate seedlot genetic worth (*GW*) and effective population size (*Ne*). The *GW* of seedlots planted within a management unit[3] contributes to estimates of timber production over time, and may be considered in timber supply analyses. *Ne* is a surrogate measure for genetic diversity, and must exceed a minimum value of 10 to meet registration requirements under the Standards.

Within any given management unit, many different seedlots from a variety of species and parent trees are planted over time. Consequently, errors in *Ne* or *GW* estimates will approach zero at the landscape level *when bias is small*. Furthermore, genetic diversity (estimated by *Ne*) at the landscape level will greatly exceed that of individual seedlots due to the diversity of seedlots on the landscape.

3.2.1 *GW* error and bias Bias will tend to be a greater problem than error when *GW* estimates are used in timber supply analyses, as they may lead to consistently over- or underestimating timber flow over time. Bias may be derived from:

1. Biased parent tree breeding value estimates:
   Breeding value estimates are the subject of considerable work (Xie and Yanchuk 2003). Possible bias associated with breeding values is beyond the scope of this paper. For this discussion, breeding values will be assumed to be unbiased (although not error-free).
2. Improper sampling procedures when estimating male or female gamete contributions (including tendencies to over- or underestimate contributions from parents with high or low breeding values in sampling):
   With proper sampling, bias is unlikely. Sampling procedures are discussed below. Avoiding sample bias and following proper procedure is the responsibility of orchard managers.
3. Unknown and persistent positive or negative correlations between parent tree breeding value and fecundity (i.e., filled seeds per cone, pollen viability, pollen competitive advantage, or competitive advantage or unintentional directional selection during nursery seedling production) (El-Kassaby and Barclay 1992; El-Kassaby and Thomson 1995; El-Kassaby 2000):

3 Area of land on which timber is grown and harvested, and for which a timber supply analysis is conducted to estimate timber flow over time under various management scenarios.

This does not include correlations between BV (Breeding Value) and the propensity of a parent to produce male or female cones, as cone production is estimated when sampling. Unknown correlations between fecundity and breeding value will occur, but are likely small at the population level. Such correlations could be either positive or negative, and are expected to depend on the sample of parents that occur in the orchard rather than on a strong population trend.

4. Improperly estimated pollen contamination levels:
   These estimates are the most likely source of error in estimating seedlot *GW*. The scale of this potential bias, however, is reduced because it only affects the male contribution to a seedlot. Due to location, most orchards in British Columbia are subject to very limited or no non-orchard pollen contamination.[4] For orchards subject to large amounts of contaminant pollen, the challenge of making reasonable estimates of the proportional amount is discussed in this paper, and remains the subject of ongoing research.

3.2.2 *Ne* estimates and bias impacts Error and bias in gamete contribution estimates from parent trees or from non-orchard pollen may result in bias in *Ne* estimates for seedlots (Xie et al. 1994). Bias in *Ne* estimates may be high or low, depending on the circumstance.

The Standards require seedlots to meet a minimum *Ne* of 10. Where *Ne* estimates are consistently underestimated, and seedlots still exceed an *Ne* of 10, the underestimates pose no problems. However, *Ne* estimates that fall below 10 are problematic. Orchard managers may address this latter issue by improving estimate methods, or by modifying seedlots to increase *Ne* (adding seed from parent trees not currently in the seedlot or by mixing with seedlots produced in different years).

If *Ne* estimates are biased to the high side (frequently overestimated), seedlots may be registered when they do not, in fact, exceed the required threshold of 10. This bias could go undetected. However, this risk is low, as most seedlots in British Columbia easily exceed the minimum *Ne* threshold, as shown in Table 1.

TABLE 1 *Percentage of orchard seedlots in storage[a] at the Provincial Tree Seed Centre by effective population size (Ne). Total number of seedlots in data set = 601 (September 2004).*

Ne	Percentage of seedlots
> 10	97
> 15	86
> 20	73
> 30	50

a Note that seedlots with an *Ne* of less than 10 are not registered for use on Crown land in British Columbia.

4 Pollen flight sampling at some north Okanagan orchard sites supports assumptions that contaminant pollen sources are sufficiently distant and phenologically removed to result in contamination of orchard seed crops. Sampling continues on several sites, and information will be reviewed from time to time to test the assumption of insignificant contamination in most orchards.

This section sets out acceptable methods for estimating the female component of gamete contributions to orchard seedlots, in increasing order of accuracy. The choice of method will vary by species, orchard operation, and the needs of customers.

The female gametic contribution of parent tree i (F_i) to a seedlot is:

$$F_i = X_i / X_n$$

where:

X_i = *the total number of filled seeds, the total number of cones, or the total weight or volume of cones harvested from all ramets of parent tree i, and*

X_n = *the total number of filled seeds, the total number of cones, or the total weight or volume of cones harvested of all ramets of all (n) parent trees, such that:*

$$X_n = \sum X_i.$$

4.1 Sampling Methods for Estimating X_i

Each of the following methods is acceptable. Viable seed production varies from cone count (Reynolds and El-Kassaby 1990; Chaisurisri and El-Kassaby 1993; El-Kassaby and Cook 1994); however, for estimating seedlot genetic worth and effective population size, these methods are considered to be acceptable based on current information. Future research and information may lead to changes to these methods.

Method F1 – Visual estimate of cone number

Visually estimate the number of seed cones on each ramet of each parent tree in the orchard before harvest. Orchard managers should periodically test visual estimates by comparing actual harvest from a small sample of trees with visual estimates.

X_i = *the total estimated number of cones from all ramets of parent tree i*

Method F2 – Measured cone volume

Measure the total volume of cones harvested from all ramets of parent tree *i*.

X_i = *the measured volume of all cones harvested from all ramets of parent tree i*

Method F3 – Cone weight

Weigh all cones harvested from all ramets of parent tree *i*. Cone weights must be taken either within 2 days of harvest, or following cone drying (recommended).

X_i = *the weight of all cones harvested from all ramets of parent tree i*

Method F4 – Cone number calculated from weight for a standard volume

Determine the number and weight of cones in a random combined sample from all ramets of each parent tree using the following standard volume of cones: 10 L for western white pine; 5 L for Douglas-fir, western larch, lodgepole pine, *Abies* spp., and *Picea* spp.; and 0.5 L for western hemlock, western redcedar, and yellow-cedar. Cone weights must be taken either within 2 days of harvest, *or* following cone drying (recommended). This will ensure that differences in moisture content will have minimal impact on results.

In addition, determine the weight of all cones from all ramets of each parent tree, and calculate the total number of cones per parent tree *i* as follows:

$$X_i = (S_i \times W_i) / WSV_i$$

where:

X_i = *the number of cones harvested from all ramets of parent tree i,*
S_i = *the number of cones counted in the standard sample volume for parent tree i,*
W_i = *the measured weight of cones harvested from all ramets of parent tree i, and*
WSV_i = *the weight of the standard sample volume of cones for parent tree i.*

Method F5 – Cone number calculated from a standard volume

Determine the number of cones in a random combined sample from all ramets of each parent tree using the following standard sample volume of cones: 10 L for western white pine; 5 L for Douglas-fir, larch, lodgepole pine, *Abies* spp., and *Picea* spp.; and 0.5 L for western hemlock, western redcedar, and yellow-cedar.

In addition, estimate crop volume from all ramets of each parent tree based on the number of sacks and knowledge of the volume per sack, and calculate the total number of cones per parent tree *i* as follows:

$$X_i = (S_i \times V_i) / SV$$

where:

X_i = *the number of cones harvested from all ramets of parent tree i,*
S_i = *the number of cones counted in the standard sample volume for parent tree i,*
V_i = *the volume of cones harvested from all ramets of parent tree i estimated using the number of sacks and knowledge of the volume per sack, and*
SV = *the standard sample volume for the species.*

Method F6 – Sample of seeds

Estimate the number of cones from all ramets of each parent tree using method F4 or F5.

Dry and tumble-extract seeds from a random sample of cones from each parent tree, using sample sizes as follows:

- Douglas-fir, western larch, western white pine, *Picea* spp., and *Abies* spp. — 20 cones; and
- lodgepole pine, western hemlock, western redcedar, and yellow-cedar — 50 cones.

Count the number of filled seeds[5] from the random sample of cones for each parent tree, and calculate the number of filled seeds for each parent tree *i*. Where large numbers of filled seed are obtained, the number of filled seeds may be estimated by measuring seed volume, counting the number of seeds in a unit of volume (i.e., 1 cc), and multiplying seeds per cubic centimetre by the volume in cc's.

$$X_i = (C_i \times FS_i) / NC$$

where:

X_i = *the number of filled seeds from all ramets of parent tree i,*
C_i = *the number of cones from all ramets of parent tree i,*
FS_i = *the number of filled seeds counted from the random sample of cones from parent tree i, and*
NC = *the number of cones in the random sample of cones.*

Method F7 – Filled seeds

Estimate the number of filled seeds from all ramets of each parent tree following seed extraction and cleaning by weighing all seed from each parent tree, weighing a sample of 100 seeds from each parent tree, and calculating the total number of seeds for each parent tree, as follows:

$$X_i = (100 \times W_i) / WS_i$$

where:

X_i = *the number of filled seeds from all ramets of parent tree i,*
W_i = *the weight of all seeds from parent tree i, and*
WS_i = *the weight of a sample of 100 seeds from parent tree i.*

5 METHODS FOR ESTIMATING MALE GAMETE CONTRIBUTIONS

This section sets out several acceptable methods for estimating the male component of gamete contributions to orchard seedlots. The choice of method will vary by species, orchard operation, and customers' needs.

Male gamete contribution estimates assume a positive correlation between pollen bud abundance and final reproductive success. This correlation will vary. More accurate estimates may be obtained through the use of isozyme or DNA markers (Schoen and Stewart 1987; Roberds et al. 1991; Burczyk and Prat 1997; Stoehr et al. 1998; Stoehr and Newton 2002). However, while these methods provide more accurate assessments, they are expensive and time consuming (see below). The proposed assessment methods presented here provide a reasonable measure of male contribution in a timely manner that is compatible with seed orchard management and seedlot registration processes.

5 Filled seeds following cleaning and empty-seed removal in an aspirator or on a gravity table.

The male gametic contribution to a seedlot from parent tree i (M_i) is calculated using the following formula:

$$M_i = Y_i / Y_n$$

where:

Y_i = *an estimate of the total volume of pollen from all ramets of parent tree i, and*

Y_n = *an estimate of the total volume of pollen from all ramets of all (n) parent trees, such that:*

$$Y_n = \sum Y_i.$$

5.1 Sampling Methods for Estimating Y_i

Each of the following methods is acceptable. Methods are listed in the order of the least to the most accurate estimate of M_i.

Method M1 – Male contribution equals the portion of ramets in the orchard

This method is acceptable for lodgepole pine, western white pine, and western redcedar orchards where more than 50% of orchard ramets are older than 15 years from grafting (or rooting).

Male contributions to a seedlot are assumed to equal the proportional number of ramets from each parent in an orchard, such that:

Y_i = *the number of ramets in the orchard from parent tree i, and*

Y_n = *the total number of ramets in the orchard.*

This method must adjust proportional ramet contributions based on ramet size, by applying a crown volume[6] adjustment to ramets such that the largest ramets would receive a weighting of 1, and smaller ramets would receive a weighting equal to their crown volume divided by the crown volume of the largest ramet. Crown volumes may be applied to ramets by age class, following calculation of crown volume for a representative sample in each age class.

Method M2 – Estimate pollen volume by partial survey

Estimate the volume of pollen cones on ramets of each parent tree i through a survey of more than 50% of all ramets in the orchard.[7] The survey must be done before spent pollen buds fall from ramets, and after any pollen picking in the orchard.

A representative sample of the orchard must be surveyed. This is best accomplished by sampling all ramets in every second row, or by sampling two of every three orchard rows.

6 Crown volume (V) may be estimated by dividing the orchard into size classes (1–4 depending upon the variation in ramet size), measuring the crown height (H) and crown basal diameter (D) of a typical tree in each size class, and using the formula $V = \frac{1}{2} H * \pi * (\frac{1}{2} D)^2$, where π = 3.141. Size-class volume estimates would then apply to each tree in the size class.

7 A visual estimate of volume may be done by estimating the number of 0.5 L containers of pollen buds on a ramet. This method will require experience with pollen picking and pollen survey.

Male contributions from each parent are calculated as follows:

$$Y_i = (PVS_i \times R_i) / SR_i$$

where:

PVS_i = *the total pollen volume estimated from sampled ramets of parent tree i,*
R_i = *the number of ramets in the orchard from parent tree i, and*
SR_i = *the number of ramets surveyed from parent tree i.*

Method M3 – Estimate pollen volume by 100% survey

Estimate the volume of pollen cones on each ramet of each parent tree *i* through a survey of all ramets in the orchard. The survey must be done before spent pollen buds fall from ramets, and after any pollen picking in the orchard. Calculate the total amount of pollen from each parent tree *i* as follows:

Y_i = *the estimated volume of pollen buds from all ramets of parent tree i.*

6 METHODS FOR ESTIMATING NON-ORCHARD POLLEN CONTRIBUTIONS

Pollen contamination from natural (non-orchard) sources may be present for some species and locations. The protocol set out here applies only to species and locations listed in Table 2. For most other species and locations, pollen monitoring studies have indicated a lack of non-orchard pollen due to sufficient geographic or phenological isolation. Therefore, non-orchard pollen is assumed to be absent, or levels are expected to be sufficiently low to have no meaningful impact on seedlot genetic worth or adaptation to the target zone.

TABLE 2 *Orchard species and locations known to be affected by significant amounts of pollination from non-orchard sources*

Species	Location
Douglas-fir	All coastal locations
Lodgepole pine	Prince George

Uncertainty exists regarding non-orchard pollen impacts on seed crops of western redcedar, Sitka spruce, and western hemlock at all coastal locations. Evidence to date suggests that levels are low, but further data collection and analysis may result in one or more of these species being added to Table 2 at a future date.

Protocols for estimating the male gamete proportion of a seedlot that is derived from non-orchard pollen are presented by Woods et al. (1996), and are recommended for use in seed orchard situations listed in Table 2.

7 METHODS FOR ESTIMATING MALE GAMETE CONTRIBUTIONS THROUGH SUPPLEMENTAL MASS POLLINATION

Supplemental mass pollination (SMP) is the process of applying viable pollen from selected parents to non-isolated receptive seed cones (Wakeley et al. 1966). SMP is done for a variety of purposes, including:

1. reducing pollen contamination,
2. reducing selfing,
3. increasing the gametic contribution from specific parent trees,
4. increasing seed set,
5. overcoming pronounced reproductive phenological differences among orchard parents, and
6. adjusting parental balance.

7.1 Protocols for SMP

Protocols for SMP are presented by Woods et al. (1996). Protocol E from that publication sets out the following requirements:

- Determine the timing of female receptivity on ramets to which pollen is being applied.
- Maintain careful records of which ramets are pollinated, and the proportion of female cones on each ramet that receive SMP. Only female cones receiving direct SMP application should be included in the SMP gamete contribution calculations.
- Test pollen viability and ensure that germination is 40% or higher, and/or electrolyte leakage is less than 40% (Webber and Painter 1995).
- Maintain records of the number of applications per ramet.
- Use a compressed-air pollinator system to apply pollen to receptive female cones.

In addition, the pollen applied to an orchard through SMP that is collected from the same orchard in the same year should not be included in male gamete contribution estimates for orchard (non-SMP) pollen.

7.2 SMP Fertilization Average Rate

Previous trials indicate success from SMP when properly applied (El-Kassaby et al. 1993). Based on experience and research, using the methods presented here, 25% of the seed from cones treated with SMP will be credited with successful fertilization from the SMP pollen.

The value of 25% success will vary greatly depending upon background pollen levels, SMP pollen viability, number of SMP applications, and application technique. Estimates of actual success are difficult and expensive to obtain. Therefore, the use of an average value is considered to be reasonable and cost-effective.

7.3 Estimating Male Gamete Contributions from SMP

The proportion of seed from any parent tree *i* in a seed orchard that has been fertilized by SMP pollen may be estimated as follows:

$$PSMP_i = 0.25\ (PP_i)$$

where:

$PSMP_i$ = *the proportion of seed from parent tree i that was pollinated by SMP pollen, and*

PP_i = *the proportion of female cones on parent tree i to which SMP pollen was applied.*

7.4 Estimating the Weighted Average Breeding Value of a Mix of SMP Pollen

Pollen applied through SMP may be mixed to increase efficiency in application, and to provide higher genetic gain and diversity in resulting seedlots. The breeding value of a pollen mix is the average breeding value of the parent trees contributing to the mix, weighted by their proportions in the mix. Protocols set out in Section 7.1 must apply.

The breeding value for an SMP pollen mix *k* is calculated as follows:

$$BVSMP_k = \sum (PM_i \times BV_i)$$

where:

PM_i = *the proportion of the pollen mix from parent tree i, and*

BV_i = *the breeding value for parent tree i.*

7.5 Estimating Seedlot Genetic Worth with SMP

The Standards set out formulae for estimating seedlot genetic worth in orchards with no SMP. These formulae are extended to allow estimates when SMP is applied under circumstances with and without pollen contamination.

7.5.1 SMP with no pollen contamination The following formula estimates seedlot genetic worth (*GW*) when SMP is applied, and there is no pollen contamination:

$$GW = \sum_i \left[(PSMP_i \times BVSMP_i) + (1 - PSMP_i) \times BV_i \times M_i + (F_i \times BV_i) \right] / 2$$

where:

GW = *the genetic worth of the seedlot,*

$PSMP_i$ = *the proportion of seed from parent tree i that was fertilized by SMP pollen,*

$BVSMP_i$ = *the mean breeding value for the SMP mix applied to ramets of parent tree i,*

BV_i = *the breeding value for parent tree i,*

F_i = *the female gametic contribution to the seedlot from parent tree i, as set out in Section 4, and*

M_i = *the male gametic contribution to the seedlot from parent tree i, as set out in Section 5.*

7.5.2 SMP with pollen contamination The following formula estimates seedlot genetic worth (*GW*) when SMP is applied, and there is pollen contamination:

$$GW = \sum_i \left[(PSMP_i \times BVSMP_i) + (1 - PSMP_i) \times PC \times BVC + \left[(1 - PSMP_i - (1 - PSMP_i) \times PC) \times BV_i \times M_i \right] + (F_i \times BV_i) \right] / 2$$

where:

GW = *the genetic worth of the seedlot,*
$PSMP_i$ = *the proportion of seed from parent tree i that was fertilized by SMP pollen,*
$BVSMP_i$ = *the mean breeding value for the SMP mix applied to ramets of parent tree i,*
PC = *the proportion of contaminant or non-orchard pollen estimated as set out in Section 6,*
BVC = *the breeding value applied to contaminant or non-orchard pollen,*
BV_i = *the breeding value for parent tree i,*
F_i = *the female gametic contribution to the seedlot from parent tree i, as set out in Section 4, and*
M_i = *the male gametic contribution to the seedlot from parent tree i, as set out in Section 5.*

8 METHODS FOR INDIVIDUAL AND/OR JOINT ESTIMATION OF PARENTAL CONTRIBUTIONS, SMP SUCCESS RATE, AND POLLEN CONTAMINATION USING GENE MARKERS

Protein and DNA gene markers provide opportunities to individually and/or jointly estimate parental (male and female) contributions, rate of SMP success, and the amount of pollen contamination from outside sources. In particular, genomic and organelle (chloroplast and mitochondria) microsatellite markers (Ritland and Ritland 2000) may be used to estimate seedlot gamete contributions. However, when these techniques are used to estimate parental gamete contributions for the purpose of seedlot registration, methodology should be discussed with appropriate staff in the Ministry of Forests and Range before starting the project. Due to the variety of techniques and statistical analysis procedures available, pre-approval on methodology by the Ministry of Forests and Range will ensure acceptance of the results for seedlot registration.

9 METHODS FOR ESTIMATING PARENTAL CONTRIBUTIONS FOR VEGETATIVE LOTS

The Standards set out formulae for estimating parental contributions for vegetative lots produced from rooting cuttings, somatic embryogenesis, or other means. Cutting material may be derived directly from parent trees, from parent trees through serial propagation, or from seedlings grown from seed produced by parent trees (donor plants[8]). Seed from parent trees may

8 Seedlings grown from seed produced by parent trees are referred to in the Standards as "donor plants."

be open-pollinated (male parent unknown), or from a controlled cross (both male and female parent known).

9.1 Cuttings from Parent Trees

The contribution of a parent tree to a vegetative lot is determined in accordance with the following formula:

$$P_i = X_i / n$$

where:

P_i = *the parental contribution from parent tree i,*
X_i = *total number of cuttings from parent tree i in the lot, and*
n = *the total number of cuttings in the lot.*

9.2 Cuttings from Donor Plants Derived from Open-pollinated Seed from Parent Trees

The contribution of a parent tree to a vegetative lot that is derived from donor plants grown from open-pollinated seed is determined in accordance with the following formula. Note that this formula only estimates P_i for the female component, and, when used for calculating *GW*, assumes that the male component of the cutting lot has a *GW* of zero.

$$P_i = D_i / 2n$$

where:

P_i = *the parental contribution from parent tree i,*
D_i = *total number of cuttings from donor plants grown from seed from parent tree i in the lot, and*
n = *the total number of cuttings in the lot.*

When using P_i for calculating *Ne* for a cutting lot derived from parent trees, the following formula should be used:

$$P_i = D_i / n.$$

9.3 Cuttings from Donor Plants Derived from Controlled-cross Seed from Parent Trees

The contribution of a parent tree to a vegetative lot that is derived from donor plants grown from controlled-cross seed is determined in accordance with the following formula:

$$P_i = (M_i + F_i) / 2n$$

where:

P_i = *the parental contribution from parent tree i,*
F_i = *the total number of cuttings from donor plants grown from controlled-cross seed in which parent i is the female parent,*
M_i = *the total number of cuttings from donor plants grown from controlled-cross seed in which parent i is the male parent, and*
n = *the total number of cuttings in the lot.*

10 METHODS FOR CALCULATING *GW* AND *Ne* WHEN COMBINING SEEDLOTS

Separate seedlots may be combined for a variety of reasons, including increasing *Ne* to exceed the minimum value of 10 as set out in the Standards, or creating a larger seedlot for operational purposes. The following protocols describe how to calculate *Ne* and *GW* when two or more seedlots are combined to a single seedlot.

10.1 Calculating Genetic Worth when Combining Seedlots

The *GW* of combined seedlots will equal the average *GW* of the individual seedlots, weighted by the proportional contribution of each seedlot based on the estimated number of seedlings that may be grown from each seedlot. Estimates of the number of seedlings that may be grown from each seedlot will use estimates of seeds per gram, germination capacity, and sowing rate (seeds per cavity) that are determined independently for each seedlot, using common methodology.

The *GW* of a combined seedlot is calculated in accordance with the following formula:

$$GW_c = \sum \{ GW_j \times S_j / \sum S_j \}$$

where:

GW_c = *the genetic worth of the combined seedlot,*
GW_j = *the genetic worth of seedlot j, and*
S_j = *the estimated number of seedlings that seedlot j could produce.*

10.2 Calculating Effective Population Size when Combining Seedlots

The *Ne* of combined seedlots will use the proportional contribution of all parent trees to the combined seedlot. The proportional contribution of each parent tree *i* to the combined seedlot is calculated in accordance with the following formula:

$$P_i = \sum_j \{ P_{ij} \times S_j / \sum_j S_j \}$$

where:

P_i = *the proportional contribution of parent tree i to the combined seedlot,*
P_{ij} = *the proportional contribution of parent tree i to seedlot j, and*
S_j = *the estimated number of seedlings in seedlot j.*

As set out in the Standards, *Ne* will be calculated as follows:

$$Ne = 1 / \sum P_i^2.$$

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APPENDIX 1 RELEVANT LITERATURE

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